The South African Journal
of
Medical Laboratory Technology

ORGAN OF THE SOCIETY OF MEDICAL LABORATORY TECHNOLOGISTS OF SOUTH AFRICA

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EDITORIAL

On being asked to describe the attitude of members of the Society of Medical Laboratory Technologists of South Africa to the Society, I would use the term "apathetic".

At a recent local meeting in Durban, a member asked why the quality of the Journal had become so poor. The answer (we are given to understand) was "The Editor has lost interest".

This may be true. It is difficult to retain interest in a journal which, although published quarterly, receives insufficient material in the quarter to permit going to press. It would be interesting to know how many papers had been submitted in the past year by the questioner of the last paragraph.

Criticism, when constructive, is welcome, but it would be well if a knowledge of the difficulties and factors concerned were attained before allowing oneself the privilege of criticism.

The question of lack of productive qualities of our members, with regard to material for the Journal, was the subject of a recent editorial. We have no intention of reiterating.

IT WOULD, HOWEVER, BE WELL TO REMEMBER: THE POOR QUALITY OF THE JOURNAL IS A REFLECTION ON THE MEMBERS OF THE SOCIETY, NOT THE EDITORIAL COMMITTEE.

REDAKSIONEEL

As gevra moet wat die houding van die lede jeens die Vereniging van Mediese Laboratorium Tegnologie van Suid-Afrika tot die Vereniging is, sou ons sê dat die uitdrukking „belanglosheid" van toepassing is.

Tydens 'n plaaslike vergadering nie lank gelede nie, het 'n lid gevra waarom die kwaliteit van die Joernal so afgeneem het. Die antwoord (so word aangedui) was dat „die Redakteur belang verloor het."

Miskien is dit juis, want dit is moeilik om belang te behou in 'n Joernaal wat, hoewel dit slegs kwartaalliks verskyn, nie genoegsame ondersteuning kry gedurende hierdie tydperk om te perse te gaan nie. Dit sal interessant wees om te verneem wat die lid wat navraag gedoen het in die afgelope jaar aangebied het vir publikasie.

Kritiek, indien opbouend, is welkom. Dit sal egter wensend wees om moeilikhede wat hier betrokke is te ken, alvorens beoordeel word.

Die gebrek aan materiaal vir publikasie van ons lede is reeds in 'n vorige uitgawe bespreek en dit is onnodig om hier te herhaal.

DIT MOET ONTHOU WORD DAT DIE SWAK GEHALTE VAN DIE JOERNAAL 'N REFLEKSIE OP LEDE VAN DIE VERENIGING IS EN NIE OP DIE REDAKSIONELE KOMITEE NIE.

THE ASSAY OF HEPARIN IN BLOOD

DAVID E. EVANS

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The difficulties attending the estimation of Heparin in blood are indicated by the large number of existing techniques. In an attempt to find a suitable method, several of the existing techniques were investigated. A method which appears to be satisfactory has been evolved by modification of two of the existing techniques, viz., the Extraction Method of Jacques[1], and the Estimation Method of Bassiouni[2].

METHODS

(a) *Reagents:*

No special materials are required. Reagents conforming to "Analar" standards are used throughout.

Phenol is liquefied in an oven at 60° C. and 80 ml. is made up to 100 ml. with distilled dater.

*Azur "A"**—100 mg. is dissolved in 100 ml. of distilled water.

Ammonium Sulphate—a saturated solution is made at room temperature and a 90% saturated solution is made up from this as required.

Bassiouni's Buffered Solvent—6 ml. are required per tube. It is made up of 2 ml. of Acetone, 2 ml. of distilled water, and 2 ml. of a Glycine-Sodium Hydroxide Buffer, consisting of 20 parts of Molar Glycine Solution, and 80 parts of normal Sodium Hydroxide Solution. The Glycine NaOH Buffer is kept as a stock solution, and the solvent is made up as required.

(b) *Extraction:*

To 2 ml. of citrated plasma are added 2 ml. of 80% Phenol. The tube is stoppered and shaken vigorously to thoroughly mix the plasma and Phenol. After standing overnight at room temperature, the tube is centrifuged at 4,000 r.p.m. for 30 minutes. The upper layer is pipetted off into a 10 ml. test tube, and the Phenol layer (lower layer) is washed with 1 ml. of normal saline. After centrifugation, the wash is added to the extract, and the combined Aqueous phase is washed three times with di-ethyl-ether, using 8 ml. each time, to remove the Phenol.

After the first wash, the tubes are left standing for from 20 to 30 minutes then centrifuged at 2,000 r.p.m. for two minutes. Using a Pasteur pipette the extract is drawn out from under the ether/Phenol layer. This procedure will not be found necessary after the second and third washes, when the ether may be drawn off by suction. The extract is then heated in an oven at 70° C. for a few minutes, to remove the

*Azur "A" (Macneal, Hopkin & Williams Ltd., Batch 00584) has proved satisfactory.

ether remaining in solution. It is important to get rid of both the Phenol and the ether, as the presence of either of these in the extract will interfere with the metachromatic reaction in the assay of the Heparin.

(c) *Assay:*

To 1 ml. of the Phenol—and ether—free extract are added 4 ml. of distilled water, followed by 0.3 ml. of Azur-"A". A blank is set up consisting of 5 ml. of distilled water and 0.3 ml. of Azur solution. The tubes are left standing overnight in a dark cupboard, after which they are centrifuged at 4,000 r.p.m. for 30 minutes. The precipitate is then washed with 5 ml. of 90% saturated Ammonium Sulphate solution, and after further centrifugation the Ammonium Sulphate solution is drawn off. It may be found necessary at this stage to remove a blue scum of dye from the walls of the tube, using a swab of cotton wool affixed to the end of a length of wire. This scum is usually more pronounced in blanks and low Heparin contents and is contributary to abnormally high blank values. The precipitate proper is dissolved in 6 ml. of Bassiouni's Buffered Solvent, and the optical density read against the blank at 520 within 15 minutes.

(d) *Calibration:*

This is carried out using known amounts of Heparin in 5 ml. of distilled water, precipitation with Azur "A", and the construction of a graph—which should be linear, passing through origin.

DISCUSSION

The advantage of this method lies in its simplicity. The lack of complicated techniques makes it suitable for routine performance. The only disadvantage is the fact that from 48 to 50 hours are required for the performance of the test.

ACKNOWLEDGMENTS

This work was carried out in the Department of Haematology, S.A.I.M.R., Johannesburg, in conjunction with the Cardio-Surgical Research Unit (Nuffield) of the University of the Witwatersrand.

I wish to thank the Director of the Institute for facilities to carry out this study. I also thank Dr. H. B. W. Greig, Head of the Department of Haematology, S.A.I.M.R., under whose guidance the work was carried out, for advice and encouragement.

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A BACKGROUND TO SEROLOGY FOR THE STUDENT MEDICAL TECHNOLOGIST

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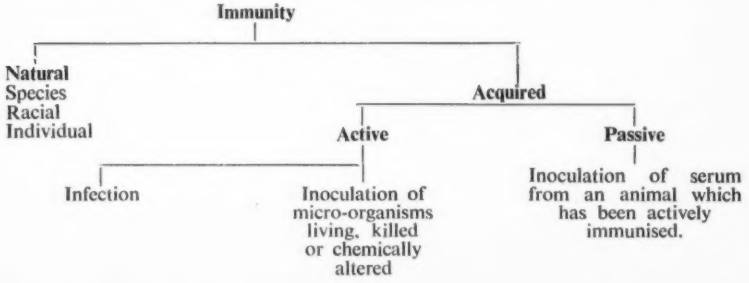
Most of our present serological techniques arise either directly or indirectly from studies of immunology. It is therefore necessary that the student have an understanding of immunity.

Immunity can briefly be considered as either the in-born or the acquired tendency of an animal to resist infection by specific micro-organisms. Immunity is a result of the natural response of the body to its invasion by micro-organisms. When the body is invaded by a micro-organism, substances are formed in the blood which help to increase the resistance of the body to infection by that specific organism. These substances are known as antibodies and generally are a tissue response to the interparental introduction of an antigen. In other words, if a person contracts measles, antibodies will be formed which will combat the disease and also tend to prevent further infection, but these actions are specific for measles and will not prevent a person contracting any other disease.

Antigen. An antigen is any substance which, when introduced parenterally into the animal tissues, stimulates the production of an antibody, and which, when mixed with that antibody, reacts specifically with it in some observable way.

Antibody. An antibody is a globulin present in the blood serum or body fluids of an animal, usually as the result of the stimulus provided by the introduction of an antigen into the animal, which reacts specifically with an antigen in some observable way.



Immunity can be divided into two parts:—

1. NATURAL IMMUNITY.
2. ACQUIRED IMMUNITY.

1. *Natural Immunity.*

Certain diseases which are common in some species of animal, are practically unknown in others closely allied to them. Thus, tuberculosis is common in pigs and cows, but rare in sheep, goats, horses and asses. Mice fall a ready prey to anthrax, while rats are unharmed. Accordingly, mice are said to be *susceptible* to anthrax while rats are said to be *immune* against it. (Species.)

In the human race, negroes are to a great extent immune to yellow fever, while the white races are susceptible. (Racial.)

Again, certain individuals escape a given disease though repeatedly exposed to infection, while others contract it. (Individual.)

This in-born tendency to resist infection with a given organism may, therefore, be characteristic of a species, a race or an individual; but it is very difficult to exclude the possibility that racial and individual immunity really belong, at least in most cases, to the acquired type. Natural immunity is seldom, if ever, absolute.

2. *Acquired Immunity.*

Acquired immunity can be divided into two types:—

(i) ACTIVE IMMUNITY.

(ii) PASSIVE IMMUNITY.

(i) *Active Immunity.*

This again can be divided into two types:—

(a) An immunity brought about through infection and

(b) ACTIVE ARTIFICIAL IMMUNITY.

(a) This form of immunity comes about through the animal contracting the disease, recovering from it and having a subsequent immunity to that particular disease. However, some diseases tend to recur again and again in the same individual. Of these, pneumonia is a prominent example. With other diseases however, to have suffered once from them is to have secured almost certain freedom from a second invasion, e.g., measles, mumps, etc. It is by no means certain how long this form of immunity lasts, but there is a good reason for believing that pneumonia is as certainly followed by a period of immunity as is smallpox, but that the immunity lasts a much shorter time.

(b) *Active Artificial Immunity.*

This form of immunity is brought about by immunisation, vaccination, inoculation, etc. Just as an attack of the disease may protect against a subsequent infection, so various methods of artificial inoculation may produce immunity. Two distinct methods of procedure can be adopted, one method being grouped under active artificial immunity while the other is grouped under passive immunity.

The micro-organisms themselves, living or dead, or some chemical product derived from them, form the material for inoculation. The result obtained in this case is comparable to that which follows actual infection with the organism in question. This form of immunity is usually of considerable though varying duration and depends upon the activity of the tissues of the recipient of the inoculation.

2 (ii). *Passive Immunity.*

The serum of an animal having undergone active artificial immunity, or the serum of a patient who has recovered from the actual infection, forms the material for inoculation. The increased resistance obtained in this case depends upon the presence in the tissues of the protective substances which existed in the serum inoculated, the tissues of the recipient playing a passive role. This form of immunity is usually of short duration.

The value of passive immunity is in the treatment of diseases which are accompanied by toxaemia, e.g., diphtheria and tetanus, and also in diseases in which the clinical course is often short, e.g., Shiga bacillary dysentery and gas gangrene, where the patient may die before any specific response has had time to manifest itself. The reason for this is that only after the first few days, following the invasion of tissue by bacteria of species of which the body has had no previous experience, have elapsed, do the earliest signs of fully specific, active, immune responses appear in the blood. Therefore, in a case where toxaemia is a danger, passive immunity alleviates the clinical manifestations evoked by the toxaemia through neutralisation of bacterial products in the blood stream before they have an apportunity of injuring vital structures.

ANTIGEN-ANTIBODY REACTIONS

Antigens and antibodies react with each other in many different ways, and it is on these different reactions, either directly or indirectly, that serological techniques are based. We will now briefly consider the following antigen-antibody reactions in turn:—

1. Precipitin reactions.
2. Toxin-antitoxin reactions.
3. Agglutination.
4. Opsonic effects.
5. Bacteriolysis.
6. Lysis of other foreign cells. (e.g., Red corpuscles.)
7. Complement fixation tests.
8. Anti-viral reactions.

1. PRECIPITIN REACTIONS.

The simplest test-tube reaction which can be studied in respect of antibody formation is that of precipitation. In the other reactions—agglutination, lysis, etc.—we are dealing with whole cells which may contain many different antigenic components; but with the precipitin reaction we can use a chemically pure single antigen in molecular or colloidal solution. If such a solution, e.g., of purified egg albumin, is injected into an animal, the animal's serum acquires the property of precipitating egg albumin when mixed with a solution of it in a test-tube. The maximum precipitation (or flocculation as it is often called) occurs when the antigen and antiserum are mixed in a certain optimum ratio and when electrolytes are present in a certain amount.

While not a precipitin reaction as such, this is probably the best place to briefly discuss haptens.

Haptens. Certain non-protein substances—e.g. lipoids, carbohydrates, etc.—though unable to act as antigens in vivo, may, when combined with a protein, be capable of inciting the formation of antibodies which in vitro react specifically with the non-protein substance.

The specific characters of the antigen of a bacterium may, in fact, depend upon a non-protein constituent—e.g., a polysaccharide as in the case of the different types of the pneumococcus and groups of haemolytic streptococci.

2. TOXIN-ANTITOXIN REACTIONS.

Exotoxins constitute a special group of bacterial antigens which are freely liberated in soluble form from the bacteria producing them both in vivo and in culture, and which, during the course of the natural disease or as a result of artificial immunisation, excite the formation of special neutralising antitoxins. The toxin-antitoxin reaction is only a special instance of precipitin reactions in general; when toxin and antitoxin are mixed in a test-tube, specific precipitation occurs. However, the precipitating antibodies which render exotoxins harmless deserve their special name "antitoxins", because the dangers of diseases

like diphtheria and tetanus depend wholly on the bacterial exotoxins. Therefore, recovery from or immunization against these diseases, depends solely on the successful development or artificial introduction of sufficient exotoxin-neutralising antibody.

3. AGGLUTINATION

Any foreign cells—bacteria, fungi, foreign red corpuscles or tissue cells—injected into an animal's tissues, will stimulate the formation of specific agglutinins, and cells of the same kind will be clumped and precipitated when mixed in a test-tube with the antiserum so produced. It is, of course, with the pathogenic bacteria that we are primarily concerned. The agglutination reaction differs from the precipitin reaction only in the larger size and greater chemical complexity of the particles of antigen. We are observing the behaviour not of protein molecules in solution, but of sensitized cells whose surfaces become blanketed by globulin antibodies. The surface of a bacterial cell usually contains multiple antigenic substances, and the injection of bacteria into an animal induces the formation of a corresponding number of distinct antibodies. However, we must note here that multiple antibodies are often concerned in the blanketing of the bacterial surface and that the main visible result of this is agglutination. Dead bacteria are agglutinated in the same way as living ones. Agglutination differs from simple precipitin reactions in that, whereas in the latter the bulk of the flocculus consists of globulin antibody, the bulk of the agglutinated flocculus consists of antigen, i.e., the bacterial cells.

FLAGELLAR AND SOMATIC AGGLUTININS

The multiplicity of antibodies evoked by bacteria, which has just been referred to, is well exemplified in the case of motile bacteria, e.g., typhoid and related bacilli. These have two distinct sets of antigenic constituents, one set in the flagella and the other in the body or somatic part of the bacteria. The flagellar antigen is called "H" antigen and the somatic is "O" antigen.

When flagellate bacteria are introduced into the body, distinct H and O agglutinins are formed. Differential testing of a serum for H and O agglutinins can be carried out by varying the condition of the bacterial suspension used in the tests, e.g., by inactivating the H antigen by alcohol or by heat, or by using a non-motile variant of the organism (devoid of flagella). The distinction between H and 0 agglutination is of practical as well as theoretical interest; the H and O results do not always run parallel, and it is usual to carry out Widal tests with standard H and O bacterial suspensions. As a rule in enteric infections, both H and O agglutinins are developed, but in some cases only one of these is demonstrable, especially in early stages of the disease. In prophy-

lactic immunization also, it has been found that the O antigens are of greater importance than the H antigens. In certain virulent freshly isolated strains of typhoid bacilli, the O antigens include a special component which is lacking in less virulent older cultures; this has been called the "Vi" antigen (Virulence antigen). It is probable that many other bacteria have similar "Virulence" antigens; the capsular polysaccharide-protein complexes of the pneumococcus are similar in nature.

GROUP AGGLUTININS

It sometimes happens that two or more related species of bacteria possess certain antigenic constituents in common, e.g., the typhoid, paratyphoid and food poisoning bacilli. Consequently, antisera prepared by using them as antigens will have common agglutinins as well as wholly specific ones; and in doing agglutination tests with these bacteria we will get some overlapping or "group agglutination".

4. OPSONIC EFFECTS

The mobile phagocytes of the human body are of two kinds, (a) the polymorphonuclear leucocytes, called by Metchnikoff MICROPHAGES, and

(b) the large mononuclear cells or MACROPHAGES, which include the monocytes of the blood and similar wandering phagocytes (histiocytes) in the tissues. In addition to these mobile scavengers, the body has large depots of fixed phagocytes, often spoken of collectively as:

(c) the RETICULO-ENDOTHELIAL system, which includes the sinus-endothelial cells of the lymph glands, spleen, liver (Küpffer's cells) and bone marrow. Foreign particulate matter in the blood stream is removed principally by these fixed phagocytes; this applies to non-living material such as carbon particles or dyes as well as to living bacteria, protozoa and viruses.

Phagocytosis is thus a non-specific process, which comes into play whenever particulate foreign matter is introduced into the tissues or fluids of the body. The phagocytosis of bacteria is not essentially different from that of inert particles; and non-pathogenic or avirulent bactria, injected into the tissues, body cavities or blood, are quickly removed by the mobile or fixed phagocytes. In the phagocytosis of pathogenic bacteria, however, specific antibodies named "opsonins", are concerned.

Opsonins are almost certainly not a distinct class of antibodies, but are the same kind of modified globulin molecules which under other circumstances may cause agglutination or bacteriolysis. How they promote phagocytosis of bacteria is not clear; they may act by neutralizing particular antigenic components of the bacterial capsules which

inhibit phagocytosis, or by damaging the capsules and liberating substances which are positively chemotactic for phagocytes, or they may act merely by blanketing the bacterial surface with a layer of innocuous protein, thus converting the bacteria into so many chemically inert particles which are then engulfed like any other inert particles.

Suffice to say that if we mix together a suspension of virulent living pneumococci with living human or rabbit leucocytes, few or none of the cocci will be phagocytosed. If to this we add serum from a healthy person or from a patient with early pneumonia, phagocytosis will not be increased. But, if we add serum from a patient who has just passed the crisis, the phagocytes will quickly engulf and digest most of the cocci. This sudden improvement in phagocytosis is clearly due to some specific substances which appear in the patient's blood at or about the time of crisis. Similar results are obtained experimentally; the sera of animals artificially inoculated with pneumococci or other virulent bacteria contain specific phagocytosis-promoting substances which the sera of untreated animals lack. These substances are called opsonins or bacteriotropins.

5. BACTERIOLYSIS

The lysis of bacteria by bacteriolysins or bacteriocidins differs from the other reactions so far considered in that it requires the presence of a normal non-specific unstable component of fresh serum called COMPLEMENT.

COMPLEMENT (or Alexine) is a thermolabile substance, or substances, present in varying concentrations in the blood serum of most normal animals, which has the property of bringing about the lysis of certain cells and bacteria, in conjunction with certain antibodies that render the cells or bacteria sensitive to its action. (This definition is incomplete, for it begs the question as to whether complement, acting by itself, produces any significant reaction whatever, and it omits reference to other effects which are probably due to the same complex.)

In bacteriolysis then, the sequence of events is this: the antibody combines firmly with the bacterial antigen, the complement then unites with the antibody-coated bacterium and lysis of the bacterium ensues.

6. LYSIS OF OTHER FOREIGN CELLS

Just as bacteria introduced into the tissues evoke the formation of specific bacteriolysins so also the artificial introduction of any other kind of foreign cells leads to the formation of specific "cytolysins". In most of the investigations in this field, the foreign cells used have been red blood corpuscles of other species. These are especially useful in such studies because they are cells of a uniform size and structure, already freely suspended in fluid, and their lysis—haemolysis—is easy

to observe because it liberates their coloured haemoglobin which dissolves in and tints the supension fluid.

The reagents necessary for haemolysis are as follows:— Washed sheep corpuscles; antibody (in this case antisheep haemolysin): and complement. When these reagents are mixed in various combinations, and the mixtures incubated at 37° C., the following results are obtained:

(1) Red cells + antibody ——> No haemolysis.

(2) Red cells + complement ——> No haemolysis.

(3) Red cells + antibody + complement ——> Complete haemolysis.

If mixtures of (1) and (2) are centrifuged, and the deposit and supernatant examined separately for the presence of antibody and complement, the following results will be noted, provided that the proportions of the reagents in the original mixtures have been suitably adjusted.

(4) Deposit from (1) + complement ——> Haemolysis.

(5) Supernatant from (1) + red cells + complement ——> No haemolysis.

(6) Deposit from (2) + antibody ——> No haemolysis.

(7) Supernatant from (2) + red cells + antibody ——> Haemolysis.

Reaction (4) shows that antibody has combined with the red cells in (1) and sensitized them to the lytic action of complement. Reaction (5) confirms this by demonstrating the absence of antibody from the supernatant fluid. Reaction (6) shows that complement has not combined directly with the red cells in (2). Reaction (7) confirms this by demonstrating the presence of complement in the supernatant fluid.

7. COMPLEMENT FIXATION TESTS

As a result of artificial immunization or natural infection, the patient's or animal's serum contains specific antibodies. These may be bacteriolytic, so that a sample of the serum will lyse a suspension of bacteria of the species that caused the infection. In the process of lysis, complement will be used up, and its partial or complete disappearance can be demonstrated by the testing of the solution, using an artificial haemolytic system as an indicator. We can thus ascertain whether complement has or has not been "fixed" (used up) as a result of the previous admixture of the patient's serum and bacteria of the species suspected of infecting him. If this serum contained specific bacteriolysins, complement will have been fixed, and the haemolytic system will therefore show no haemolysis or diminished haemolysis. If the patient's serum contained no specific antibodies, complement will not have been absorbed and the system will undergo normal haemolysis. Sometimes specific antibodies may not be demonstrably haemolytic, yet

along with antigen they may absorb complement; and the presence of complement-fixing antibodies, tested for with a haemolytic system, is also good evidence of the identity of the bacterium causing the infection.

8. ANTI-VIRAL REACTIONS

The sera of men or animals immunized naturally or artificially against viruses, exhibit properties generally similar to antibacterial sera. With various anti-viral sera, specific agglutination, precipitation and complement fixation reactions have been observed. The inactivation of a virus by an antibody has been attributed to *viricidal* antibodies, but apparently complement is not necessary for this effect and its exact nature is still uncertain.

Addendum.

It must be remembered that, in some instances, the occurrence of antibodies may have no aetiological significance. Thus, in typhus fever a serum-antibody is demonstrable which is specific for a particular type of B. proteus although this organism has no aetiological relationship to the disease.

Antibodies like those present in typhus fever, which appear to be specific for an antigen which has no biological relationship to the antigen constituting the immunizing stimulus, are designated "Heterophile". These heterophile antibodies are of great value in the laboratory diagnosis of certain diseases, particularly syphilis, typhus fever and infectious mononucleosis.

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ABSTRACT

Method of stabilising antigen emulsion used in V.D.R.L. Syphilis tests.

Bossak, H. M., and Duncan, W. P.
Public health reports 73-9-836.

Benzoic acid is used as a stabilizing substance to preserve the reactivity level of VDRL emulsions. Parallel tests over 570 sera indicated that reactivity was not affected and that emulsions could be prepared and kept for use for periods up to six weeks.

G.W.W.

COLOUR IN THE LABORATORY

Part 1.—Introduction to Colour.

From the moment that we are aware that we are alive, from the time that we open our eyes and see the confusion of light and colour that is our world, from then colour is a major part of our lives. Most of us pass the day without a second thought for colour, it is our heritage, we were born with the ability to perceive it, therefore it is usually ignored.

The most remarkable point about colour, is our ability to see it. The average human eye can distinguish between a wavelength of 2mu, which over the visible sectrum of 360mu means, that we could with training separate 180 different hues.

It is possible to increase your colour perception by training your brain to understand more fully, the signals from your eyes. It must be understood that eyes differ in their ability and sensitivity to colour, but you can be sure that unless you have been trained, or have trained yourself, your ability to see colour can be greatly improved.

In order to understand colour it is necessary to delve a little into the science of radiation. Colour is energy, difference in colours is a difference in the degree of energy, within the wavelength scale that we call the Visible Spectrum. That portion of the total electromagnetic spectrum that our eyes can use, is but a small fraction of the whole. This ranges from radiation on sub-atomic levels (cosmic rays), to that of the commercially produced electricity supplies (a fifty or sixty cycle A.C. current). The variation in wavelength, from cosmic rays at 3.2×10^{-12} cm. (0.00000000000032 cm.) to A.C. fifty or sixty cycle current at 5×10^{8} cm. (500,000,000 cm.).

Of the visible spectrum the violet is the high energy end and the red the low energy end, the wavelength ranges from 360mu to 720mu respectively.

These figures have little meaning for us, we cannot visualise what colour is stimulated by a wavelength of 420mu, it is necessary for us to have different basis for comparison.

Colour does not begin at the violet and end at the red end of the spectrum, it takes rather the form of a wheel, the red and violet merging to give us a purple. Therefore colour has no beginning and no end, each colour merges with any combination of other colours giving a great number of hues.

In addition to the hue, we have two other factors to take into consideration when we talk of colour, they are Value and Intensity, these are the three dimensions of colour. To give them meaning we will define each separately. *Hue* is the name of the colour.

Value is its lightness or darkness, the ability to reflect or absorb light.

Intensity is its degree of purity or greyness. We all understand what is meant by Hue, however Value and Intensity need some clarification.

Picture a red paint of a certain hue, it is made up in two forms, one is of maximum hue, the other has nine-tenths of its volume in white paint added, the white paint being neutral has not in any way affected the colour, but it has altered its value or lightness. It is possibly by altering the ratio of white paint, to alter the brightness of the hue.

Intensity, the purity of a hue, depends on the amount of neutral grey *of the same value* with which it is mixed. Imagine a series of greys from pure white to absolute black, take the number of steps as being nine, these are the *value* steps. We use the middle one, number 5, we also take a green of the same value of brightness. Now, if we mix the two, the grey and green, starting with pure grey, and varying the ratios 1 : 4, 2 : 3, 3 : 2, 4 : 1 and pure green, there we have grey varying over five steps to pure green. We give these steps numbers, grey = 0, ratio 1 : 4 = /2, ratio 2 : 3 = /4, ratio 3 : 2 = /8, pure green = 0.

It is now possible to describe any colour by giving its hue, value and intensity in this manner : Hue/Yellow, Value6/, Intensity/8 or more simple Yel:6/8.

It is very difficult describing a subject graphically which should be explained pictorially; however, a little thought, and perhaps experiment, will clarify greatly your ability to understand colour. In part two of Colour in the Laboratory, what has been explained in part 1 will be applied in colorimetry.

THE BULAWAYO AND DISTRICT BLOOD TRANSFUSION SERVICE

Applications are invited for a Medical Laboratory Technologist, for The Bulawayo and District Blood Transfusion Service, Bulawayo, Southern Rhodesia. The candidate must be an F.I.M.L.T. in Haematology and have had practical experience in blood transfusion work. Starting salary will depend on age and experience but will not be less than £1,220 per annum. Further particulars from Secretary, P.O. Box 2010, Bulawayo.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG ORAL AND DENTAL HOSPITAL LABORATORY ASSISTANT, GRADE II.

Applications are invited from suitably qualified persons for appointment as Laboratory Assistant, Grade II, on the staff of the University's Oral and Dental Hospital. Applicants should have had experience of Histological methods as well as of general laboratory management. Duties are to be assumed on 1st January, 1959, or as soon as possible thereafter.

Salary will be according to the scale £550 x £50—£750 per annum, plus a temporary cost of living allowance in the case of a married man at the present rate of £234 per annum. The initial salary will be determined in accordance with the qualifications and experiences of the successful candidate. Membership of the Provident Fund is compulsory and involves a contribution at the rate of 7% of the salary. Membership of the Staff Medical Aid Fund is compulsory in the case of an officer who is eligible in accordance with the rules.

Applications, stating age, qualifications, experience, marital status and enclosing copies of recent testimonials, should be lodged with the Registrar, University of the Witwatersrand, Milner Park, Johannesburg, not later than 28th November, 1958.

NOTICE TO CONTRIBUTORS

All contributions are to be addressed to:—The Editor, The South African Journal of Medical Laboratory Technology, Room 213, Dept. of Pathology, Medical School, Umbilo Road, Durban.

Contributions may be written in English or Afrikaans, and should preferably be typed in double-spacing on foolscap sheets on one side of the paper only.

Figures should be drawn in Indian ink, and all figures and tables should be labelled as such (e.g. Figure 1, Table 1, etc.).

Authors should make adequate references to previous works on their subjects. These should be set out as follows:—Author's surname and initials of Christian names; the year of publication (in parentheses); the name of the journal, which should be abbreviated according to the World List of Scientific Periodicals (see below); the volume number (underlined); and the first page reference.

Example:—Moron, I. B. (1960). J. unsuccess. Med., 20, 99. References to books should give the author's name and initials, the year of publication, title of book, name of publisher, and town in which published.

References should be arranged in alphabetical order of the authors' surnames. If more than one work by the same author is listed, these should appear in chronological order.

Technologists are reminded that regulations demand that all original articles of a technical or scientific nature must be approved by the heads of their departments before being submitted for publication.

Title abbreviations according to World List of Scientific periodicals

All nouns commence with capital letters, and adjectives small letters. Articles, conjunctions and prepositions are omitted.

Examples:—

J. Amer. med. Ass.	*S. Afr. J. clin. Sci.*
Lancet	*Stain Tech.*
Amer. J. clin. Path.	*J. Bact.*

REPRINTS AND PHOTOGRAPHS

If requested before publication, 24 reprints of original articles will be supplied free to contributors. As a temporary measure, contributors are asked to defray the costs of publishing diagrams and photographs accompanying articles.

KENNISGEWING AAN INSENDERS

Alle bydrae moet as gevolg geadresseer word: Die Editeur, Die Suid Afrikaanse Joernal van Mediese Technologie, Kamer 213, Dept. van Patologie, Mediese Skool, Umbiloweg, Durban, Natal.

Bydrae mag in Engels of Afrikaans geskryf word en moet verkieslik getik wees dubbel spasiering op folio-papier en net op een kant van die vel.

Figure moet in Indiese ink geteken word en alle figure en tabelle moet geëtikeer word as sulks (b.v. Figuur 1, Tabel 1, ens.).

Auteurs moet voldoende referensies gee tot vorige werke oor hulle onderwerpe. Die moet as volg uiteengesit word:—Auteur se familienaam en voorletters; die jaar van uitgawe (in hakies); die naam van die Joernaal, wat moet verkort volgens die Wêreld Lys van Wetenskaplike Tydskrifte (sien hieronder)r die volume nommer (onderstreep); en die eerste pagina referensie.

Voorbeeld:—Moron, I. B. (1960). J. unsuccess. Med., 20, 99. Referensies tot boeke moet die auteur se naam en voorletters meld, die jaar van uitgawe, titel van boek, naam van uitgewer, en stad waar dit gepubliseer is.

Referensies moet in alfabetiese orde, volgens auteurs se familiename gerangskik word. Indien meer dan een werk deur dieselfde auteur gemeld word, moet dit in tydsorde voorkom.

Tegnoloë word daaraan herinner dat regulasies vereis dat alle oorspronklike artikels van tegniese of wetenskaplike aard moet die goedkeuring dra van hulle departementale hoofde voor dit ngestuur word vir publikasie.

Titel verkortings volgens Wêreld Lys van Wetenskapliker Tydskifte

Alle selfstandige naamwoorde moet begin met hoofletters en byvoeglike naamwoorde met klein letters. Artikels, verbindings, en voorsetsels word uitgelaat.

Voorbeelde:—

J. Amer. med. Ass.	*S. Afr. J. clin. Sci.*
Lancet	*Stain Tech.*
Amer. J. clin. Path.	*J. Bact.*

HERDRUKKE EN FOTOGRAWE

Indien aanvraag ingedien word voor publisering, sal 24 herdrukke van oorspronklike artikels vry aan beydraers verskaf word. As in tydelike maatreel word bydraers gevra om die koste van publisering van fotos en tekeninge wat saam met artikels gaan selt te betaal.